

Western blot analysis of cultured fibroblasts

PL Philip S. Low

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 An abbreviated version of this protocol was published in Science Translational Medicine in Oct 2020

Targeted inhibition of PI3 kinase/mTOR specifically in fibrotic lung fibroblasts suppresses pulmonary fibrosis in experimental models

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Detailed protocol

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Serum-starved confluent HLF cells were coincubated in medium containing TGFβ1 (10 ng/ml) with or without the indicated concentrations of PI3K inhibitors for 24 hours. Cells were harvested and lysed for Western blot analysis. After sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis and blocking, membranes were incubated with antibodies to detect pSMAD2Ser465/467 (Cell Signaling Technology, #3101) or pAktSer473 (Cell Signaling Technology, #4060) or pS6 Ser235/236 (Cell Signaling, #4858S) and signals were visualized with ECL Western Blot Detection Reagents (GE Healthcare). After stripping, membranes were blocked and reprobed with antibodies specific for total SMAD2 (Cell Signaling Technology, #3103) or total Akt (Cell Signaling Technology, #4060).

How to cite: (Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Low, P. (2020). Western blot analysis of cultured fibroblasts. Bio-protocol Preprint. bio-protocol.org/prep647.
2. Hettiarachchi, S. U., Li, Y., Roy, J., Zhang, F., Puchulu-Campanella, E., Lindeman, S. D., Srinivasarao, M., Tsoyi, K., Liang, X., Ayaub, E. A., Nickerson-Nutter, C., Rosas, I. O. and Low, P. S. (2020). Targeted inhibition of PI3 kinase/mTOR specifically in fibrotic lung fibroblasts suppresses pulmonary fibrosis in experimental models. Science Translational Medicine 12(567). DOI: [10.1126/scitranslmed.aay3724](https://doi.org/10.1126/scitranslmed.aay3724)

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